Kidney International, Vol. 46 (1994), pp. 886–892

Screening and confirmatory testing of cadaver organ donors for hepatitis C virus infection: A U.S. National Collaborative Study

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Screening and confirmatory testing of cadaver organ donors for hepatitis C virus infection: A U.S. National Collaborative Study. Hepatitis C virus (HCV) can be transmitted by organ transplantation. Cadaver organ donors are screened for HCV infection by testing for antibodies to HCV (anti-HCV). The prevalence of HCV infection and performance of anti-HCV tests in detecting HCV infection in organ donors are unknown. Sera from 3078 cadaver organ donors were tested for anti-HCV by a first generation enzyme-linked immunosorbent assay (ELISA1). Sera from all 137 ELISA1 positive donors and a random sample of 92 ELISA1 negative donors were tested for anti-HCV by a second generation ELISA (ELISA2) and for HCV RNA by the polymerase chain reaction. Organ bank records were reviewed for risk factors associated with HCV infection. Follow-up was available on 70 recipients of organs from 42 ELISA2 positive donors. The prevalence of HCV RNA, extrapolated to all 3078 donors, was 2.4%. Liver disease, anti-HCV and HCV RNA were detected more frequently among recipients of organs from ELISA2 positive donors with HCV RNA than from ELISA2 positive donors without HCV RNA. Among donors, the sensitivity and negative predictive value of the ELISA2 for HCV RNA were 100%. However, despite a specificity of 98.1%, the positive predictive value was only 55.1%. Clinical and laboratory characteristics did not distinguish ELISA2 positive donors with and without HCV RNA. The presence of serum HCV RNA in organ donors predicts the risk of transmission of HCV infection. Discarding organs from ELISA2 positive donors would eliminate transmission, but organs from 1.88 percent of donors would be wasted. To reduce waste, it is necessary to develop confirmatory tests with a higher specificity for HCV RNA than those that are currently available.

Liver disease due to non-A, non-B hepatitis is an important cause of morbidity and mortality in renal transplant recipients [1–3]. We have previously shown that hepatitis C virus (HCV), the principal cause of non-A, non-B hepatitis, can be transmitted by organ transplantation [4, 5]. In our study, post-transplantation liver disease was found in 48% of recipients of organs from cadaver donors with antibody to HCV (anti-HCV) [4]. Based on these findings, several organ procurement organizations have adopted a policy restricting the use of anti-HCV positive donors to life-saving transplants (heart, liver or lung) [4–6]. A similar policy has been recommended by the U.S. Public Health Service Inter-Agency Guidelines [7]. However, others have reported a lower prevalence of liver disease among recipients of organs from anti-HCV positive donors [8–10]. Because of the conflicting data, some authors have argued against a moratorium on renal transplantation from organ donors with a positive anti-HCV test until more information is available regarding the prevalence of viremia among cadaver organ donors, and the performance of anti-HCV tests in detecting donors at risk of transmitting the virus [11].

The tests currently used to screen cadaver organ donors detect antibody to HCV but not HCV itself. Consequently, both false negative and false positive tests can occur, which would lead to transmission of HCV infection and wastage of uninfected organs, respectively. The polymerase chain reaction (PCR) has been used to detect HCV RNA in the serum of anti-HCV positive organ and blood donors [5, 6, 12, 13]. A positive test is associated with uniform transmission of infection [5, 6, 12, 13]. However, PCR is not practical for use in anti-HCV positive cadaver organ donors [14], and hence, other confirmatory tests are required. Therefore, we initiated a national collaborative study to examine the prevalence of HCV infection among cadaver organ donors, confirm the effect of viremia on the risk of transmission, determine the sensitivity and specificity of clinical and laboratory tests in identifying donors with HCV infection, and evaluate strategies for screening and confirmatory testing of donors for HCV infection.

Methods

Analysis plan

(1) To determine the prevalence of HCV infection among cadaver organ donors, we analyzed sera from a sample of anti-HCV positive and anti-HCV negative donors for HCV RNA by PCR. In this analysis, donors were initially classified by the results of a first generation enzyme-linked immunosorbent assay (ELISA1) because it was commercially available at the time of

Received for publication February 23, 1994
and in revised form April 6, 1994
Accepted for publication April 7, 1994

Table 1. Prevalence of markers of HCV infection among cadaver organ donors

Organ procurement organization	Study period	Donors tested[a]	Prevalence of HCV infection		
			ELISA1	ELISA2[b]	PCR[b]
New England Organ Bank, MA	1986–92	1012	2.9%	2.4%	1.7%
Regional Organ Bank of Illinois, IL	1987–91	596	6.0%	3.2%	2.7%
Lifelink Inc, FL	1985–91	521	7.7%	7.3%	4.0%
Center for Organ Recovery and Education, PA	1986–90	516	5.8%	5.4%	2.1%
Midwest Organ Bank, KS	1988–90	172	4.7%	4.1%	2.0%
Louisiana Organ Procurement Agency, LA	1990–91	129	2.3%	2.3%	0.8%
Oregon Health Sciences University, OR	1990–91	67	1.5%	NA	NA
Washington Hospital Center, D.C.	1991	66	16.7%	8.3%	4.2%
Total		3078	5.1%	4.2%	2.4%

[a] Tested by ELISA1 at participating OPOs, one donor was common to two OPOs

[b] Extrapolated to the donor population

initiation of the study. (2) To determine the sensitivity and specificity of newer anti-HCV tests for detection of HCV infection, sera from ELISA1 positive and ELISA1 negative donors were tested with other currently available assays. Because of the limited volume of sera available from some donors, not all sera were tested by all assays. (3) To confirm the effect of viremia on the risk of transmission, we compared the prevalence of post-transplantation liver disease and markers of HCV infection among recipients of organs from anti-HCV positive donors with and without serum HCV RNA. In this analysis, donors were classified by a second generation ELISA (ELISA2) because it is the current screening test for HCV infection. (4) To determine the risk factors for anti-HCV among cadaver organ donors, we compared the clinical and laboratory characteristics of anti-HCV positive and anti-HCV negative donors. In this analysis, donors were classified by ELISA1 because it was the screening test used to select them. (5) To determine whether clinical and laboratory tests could differentiate between anti-HCV positive donors with and without current HCV infection, we compared these parameters in anti-HCV positive donors with and without HCV RNA. This analysis was restricted to ELISA2 positive donors. (6) To assess the performance of screening and confirmatory tests, we calculated the predictive values of testing strategies to detect HCV infection. A high negative predictive value (NPV) maintains a low rate of transmission of HCV infection by infectious donors, while a high positive predictive value (PPV) reduces wastage of organs from non-infectious donors.

Study population

Selected organ procurement organizations (OPO) with facilities for retrieval of archived and prospectively collected sera from organ donors were invited to participate in this study (Table 1).

Cadaver organ donors. Prospectively collected or stored sera (−70°C) from 3078 cadaver organ donors over a seven year period (1986–92) were tested for anti-HCV at the individual OPOs by ELISA1. One hundred and fifty-seven donor sera (5.1%) tested positive and were included in the study. Organ bank records and sera were available in 149 and 137 of the ELISA1 positive donors, respectively. One hundred randomly-selected anti-HCV negative cadaver organ donors served as controls. The number of anti-HCV negative controls from each OPO included in the study was proportional to the number of anti-HCV negative donors at each OPO. Within each OPO, the anti-HCV negative controls were randomly selected. Organ bank records and sera were available in 94 and 92 of the anti-HCV negative controls, respectively. All anti-HCV positive donors and anti-HCV negative controls were also tested for HCV RNA by PCR and for anti-HCV by ELISA2, and if sufficient sera were available, by a second generation recombinant immunoblot assay (RIBA2).

Recipients of organs from anti-HCV positive donors. In three OPOs (New England Organ Bank, Midwest Organ Bank and Lifelink Foundation Inc.), follow-up was available on 70 recipients of organs from 42 anti-HCV (ELISA2) positive donors in whom sera were available for testing for HCV RNA. In association with their treating physicians, pre-transplantation and post-transplantation records were reviewed (by BJGP, CFB or CEW, respectively). In surviving recipients, tests for HCV infection were performed on serum samples obtained at the most recent follow-up visit. In patients who had died and in whom stored sera were available, a test for anti-HCV was performed on the most recent sample prior to death. Results on some of these recipients have been previously reported [4–6].

Definitions

Liver disease is defined as an increase in serum levels of alanine aminotransferase (ALT) to more than 2.5 times the upper limit of normal (Normal <40 U/liter) [15]. Acute liver disease is defined as an elevation in the serum ALT on two or more occasions at least two weeks apart but for less than six months. Chronic liver disease was defined as a persistent elevation of ALT for more than six months [15]. Sub-fulminant liver failure is defined as onset of hepatic encephalopathy between two weeks and three months after the onset of jaundice [16].

Laboratory tests

Donor sera were tested for anti-HCV by ELISA1 at the OPOs. Samples were tested for anti-HCV by ELISA2 at the State Laboratory Institute, Jamaica Plain, Massachusetts, USA and by RIBA2 at the Diagnostics Development Department, Chiron Corporation, Emeryville, Ca. The first generation enzyme-linked immunosorbent assay, ELISA1 (Ortho Diagnostic Systems, Raritan, New Jersey or Abbot Laboratories, Abbot Park, Illinois, USA) detects antibody to a recombinant HCV antigen (c100) from the non-structural region of the HCV genome. The second generation enzyme-linked immunosorbent assay, ELISA2 (Ortho Diagnostic Systems, Raritan, New Jersey, USA) detects antibody to recombinant HCV antigens derived from three regions (c100, c200 and c22) from the non-structural and core regions of the HCV genome. Both assays were performed according to manufacturer's instructions. The second generation recombinant immunoblot assay, RIBA2 (Chiron Corporation, Emeryville, California, USA), a strip immunoblot assay, detects antibody to four recombinant HCV antigens (5-1-1, c100 c33, c22) from the non-structural and core regions of the HCV genome and is currently recommended as a confirmatory test [17, 18]. However, both positive and negative sera by the ELISA1 were tested by this

assay. Tests were carried out according to the manufacturer's instructions, and classified as positive, negative or indeterminate. For purposes of analysis, indeterminate tests in this high risk population were considered positive.

Samples were tested for HCV RNA using the reverse transcriptase polymerase chain reaction (RT-PCR) at the San Francisco Veterans Administration Medical Center, San Francisco, California, USA [19, 20]. All PCR positive samples and ten randomly selected PCR negative samples were retested for HCV RNA by RT-PCR at a second laboratory (Palo Alto Veterans Administration Medical Center, Palo Alto, California, USA). Only samples that tested positive for HCV RNA at both laboratories were considered positive. To avoid contamination of samples, nucleic acid extraction and genomic amplification steps were performed in separate laboratories [21]. Samples were tested in duplicate and repeated if discrepant results were obtained. Only repeatedly positive reactions were considered positive. Serum samples were coded so that the laboratory personnel could not distinguish the source, and personnel at each laboratory were blinded to the results of the other assays to prevent bias.

Calculations and statistics

The **Appendix** shows the equations for calculation of (1) prevalence of HCV infection among cadaver donors; (2) sensitivity, specificity, PPV and NPV of various anti-HCV tests; and (3) transmission of HCV and waste of donor organs for different strategies for screening and confirmatory testing. The relationship between risk factors and test results were assessed by Pearson's statistic for the χ^2 test (discrete variables) and the Wilcoxon two-sample test (continuous variables) using two-sided P values. Since the data were sampled as in a case-control study, we measured the effect of a two-level risk factor by the odds ratio and calculated 95% confidence intervals using the standard asymptotic formula for the variance of the odds ratio [22]. Samples missing values for a particular risk factor were excluded from analysis of that factor. Concordance between tests was measured by the kappa statistic. Contingency table analysis and Wilcoxon tests were run in SAS/Stat (SAS Institute Inc., Cary, North Carolina, USA).

Results

Prevalence of anti-HCV and HCV RNA among cadaver organ donors

Anti-HCV was detected by ELISA1 in 157 (5.1%) of 3078 cadaver organ donors at the eight OPOs (Table 1). The prevalence of a positive test for ELISA1 ranged from 1.5% to 16.7% in different OPOs. Anti-HCV was detected by the ELISA2 in 110 of 134 (82.1%) ELISA1 positive donors and none of 92 (0%) ELISA1 negative controls. Therefore, the prevalence of ELISA2, extrapolated to all 3078 cadaver organ donors, was 4.2% with a range from 2.3% to 8.3% among OPOs. HCV RNA was detected by PCR in 64 of 137 (46.7%) ELISA1 positive donors and none of 92 (0%) ELISA1 negative controls. Therefore, the prevalence of HCV RNA, extrapolated to the cadaver organ donor population, was 2.4% with a range from 0.8% to 4.2% among OPOs.

Recipients of organs from ELISA2 positive donors with and without HCV RNA

Follow-up was available on 70 recipients of organs from 42 ELISA2 positive donors in whom sera were available for testing for HCV RNA (Table 2). Forty-seven recipients received organs (37 kidneys, 7 hearts and three livers) from 26 ELISA2 positive donors who tested positive for HCV RNA. The remaining 23 recipients received organs (21 kidneys and two hearts) from 16 ELISA2 positive donors who tested negative for HCV RNA. Post-transplantation liver disease was observed in 22 of 47 (47%) recipients from ELISA2 positive donors with HCV RNA compared to five of 23 (22%) recipients from donors without HCV RNA (P = 0.04). The post-transplantation prevalence of a positive ELISA2 and HCV RNA was significantly greater in recipients of organs from ELISA2 positive donors with HCV RNA compared to recipients from donors without HCV RNA. Among recipients of organs from ELISA2 positive donors with HCV RNA, the post-transplantation prevalence of HCV RNA was 100% (26 of 26 recipients). These data confirm the importance of serum HCV in ELISA2 positive donors in predicting transmission of HCV infection.

Table 2. Prevalence of post-transplantation liver disease, markers of HCV infection and outcome in recipients of organs from ELISA2 positive donors with and without HCV RNA

Recipient characteristics	Recipients of ELISA2 positive donors: PCR positive N = 47	PCR negative N = 23	P value
Organ			
Kidney	37	21	
Heart	7	2	0.33
Liver	3	0	
Mean follow-up ± SD *months*	16 ± 11	24 ± 10	0.11
Post-transplant liver disease	22/47 (47%)	5/23 (22%)	0.04
Type			
Acute	5/22 (23%)	0/5 (0%)	
Chronic	16/22 (73%)	5/5 (100%)	0.42
Sub-fulminant	1/22 (4%)	0/5 (0%)	
Cause			
Non-A, non-B hepatitis	21/22 (95%)	5/5 (100%)	
Other	1/22 (5%)	0/5 (0%)	0.63
Post-transplant			
ELISA1 positive	12/33 (36%)	3/12 (25%)	0.47
ELISA2 positive	19/26 (73%)	2/8 (25%)	0.01
RIBA2 positive	16/26 (62%)	2/8 (25%)	0.07
PCR positive	26/26 (100%)	3/7 (43%)	<0.001
Deaths	8/47 (17%)	3/23 (13%)	0.64
Graft loss	12/47 (26%)	3/23 (13%)	0.23

Estimated rates of transmission of HCV and donor wastage using current screening tests

Based on the detection of HCV RNA by PCR, the sensitivity of both ELISA1 and ELISA2 was 100% (Table 3). The NPV of each test was also 100%. The specificity of ELISA1 was 97.2% (the range among OPOs was 87.0% to 98.8%) and the specificity of ELISA2 was 98.1% (range 95.7 to 99.3%). The PPV of ELISA1 was 46.7% (range 25.0 to 59.3%) and the PPV of ELISA2 was 55.1% (range 33.3 to 80.0%). Based on these estimates, discarding organs from ELISA2 positive donors would eliminate transmission of infection, but non-life saving organs (kidney, pancreas) from 1.88% of donors would be wasted.

Table 3. Proposed testing for screening and confirming HCV infection among cadaver organ donors

Test	Sensitivity	Specificity	NPV	PPV	Transmission donor waste
	%				
Screening tests					
ELISA1	100.0	97.2	100.0	46.7	0.00	2.72
ELISA2	100.0	98.1	100.0	55.1	0.00	1.88
Confirmatory tests						
ELISA2 optical ratio >3.0[a]	98.3	23.9	91.7	62.0	0.04	1.43
RIBA2 positive[a]	100.0	6.8	100.0	41.4	0.00	1.76

[a] Among ELISA2 positive donors

Comparison of clinical and laboratory characteristics of anti-HCV (ELISA1) positive and negative donors

Male gender, history of alcohol abuse, history of drug abuse, blood alcohol levels greater than 100 mg/dl, presence of drugs in the blood with potential for abuse, and presence of anti-HBc or anti-CMV were significant risk factors for anti-HCV (Table 4). The same characteristics were also risk factors for anti-HCV by ELISA2 (data not shown). The concordance (κ statistic) between ELISA2 and RIBA2 was 96% ($N = 148$).

Comparison of clinical and laboratory characteristics of anti-HCV (ELISA2) positive donors with and without serum HCV RNA

Among ELISA2 positive donors, the only risk factor for HCV RNA was an ELISA2 optical ratio (OR) > 3.0 (Table 5). However, there was large overlap between OR results in HCV RNA positive and negative patients (data not shown). A positive RIBA2 was not a risk factor. These data indicate that risk factors for HCV infection do not distinguish anti-HCV positive donors with and without ongoing HCV infection as shown by serum HCV.

Estimated rates of transmission of HCV and donor wastage with proposed confirmatory tests

We evaluated the performance of a positive RIBA2 and an ELISA2 or >3.0 as possible confirmatory tests in organ donors that test positive by ELISA2 (Table 3, lower panel). Extrapolating to the entire donor population, using RIBA2 as a confirmatory test in ELISA2 positive donors would maintain transmission at zero and reduce wastage to 1.76% of donors. Using the ELISA2 OR > 3.0 as a confirmatory test would permit transmission of infection from 0.04% of donors and further reduce wastage to 1.43% of donors.

Discussion

The use of PCR, with primers directed to the highly conserved 5′ untranslated region of the HCV genome, has provided a highly sensitive tool for the detection of HCV RNA [23–31]. Indeed, HCV RNA has been detected in the majority of high-risk anti-HCV positive individuals, such as blood donors with elevated serum alanine aminotransferase levels, intravenous drug abusers, hemophiliacs and patients with non-A, non-B hepatitis [26–30]. Further, HCV RNA is rarely detected in low-risk populations with a negative anti-HCV test [26]. Therefore, we used the PCR as the "standard" to denote the presence of persistent "productive viral infection" [24]. Our results confirm that detection of serum HCV RNA by PCR in cadaver organ donors indicates a high risk of transmission of HCV infection: 100% of recipients tested positive for HCV RNA after transplantation and 47% of recipients developed post-transplantation liver disease. Therefore, it is essential to test organ donors for HCV infection.

We observed a 2.4% prevalence of serum HCV RNA among cadaver donors, with a range from 0.8% to 4.2% among the eight OPOs that participated in this study. Because of the low prevalence of HCV infection, the PPV of the ELISA1 and ELISA2 anti-HCV tests were only 46.7 and 55.1%, respectively, with a broad range among OPOs. We suggest that differences in the prevalence of donor HCV infection and in the PPV of anti-HCV tests at different OPOs may account for the reported differences in the prevalence of post-transplantation liver disease and markers of HCV infection among recipients of organs from anti-HCV positive donors [4–10]. Further, our data indicate that the presence of liver disease or a positive test for anti-HCV does not identify all recipients with HCV infection. Failure to test recipients for HCV RNA underestimates the prevalence of HCV infection, and may be another explanation for the differences in post-transplantation HCV infection observed at different OPOs [8, 9].

The absence of HCV RNA in a significant proportion of anti-HCV positive organ donors in this study could be due false positive tests for anti-HCV, false negative tests for HCV RNA, or persistent antibody following resolution of infection. The similarity of clinical features among ELISA2 positive donors with and without HCV RNA suggests that the ELISA2 positive donors without HCV RNA were not likely to be false positive. Rather, it is more likely that they had indeed been exposed to HCV and either cleared the virus or had low levels of viremia that could not be detected by PCR. The cadaver donor sera tested in this study were stored for as long as seven years, and it is possible that prolonged storage and frequent freeze-thaw cycles may have resulted in a degradation of HCV RNA. Indeed, Busch et al have shown that imperfect handling and/or storage of blood samples can lead to failure to detect HCV RNA in almost 40% of samples [24]. These authors suggested that the low rate of detection of HCV RNA in anti-HCV positive blood donors in some studies [13, 26, 31] could reflect false negatives due to poor handling and storage of test samples. We too observed five patients with post-transplantation liver disease who received organs from ELISA2 positive donors without HCV RNA. However, of these five patients, the single patient in whom pre-transplantation serum was available for testing had pre-transplantation HCV infection. Moreover, the significant differences in the prevalence of post-transplantation liver disease and HCV RNA among recipients of organs from ELISA2 positive donors with and without HCV RNA suggest that most of the HCV RNA negative donors were truly negative.

None of the anti-HCV negative organ donors in this study tested positive for HCV RNA (sensitivity was 100%). Consequently, the NPV was 100%, and transmission of HCV infection by anti-HCV negative donors would be extremely unlikely. Therefore, using the ELISA2 anti-HCV test to screen organ donors could virtually eliminate transmission of HCV infection. The specificity of the anti-HCV tests was also high. However, because of the low prevalence of infection, the PPV of ELISA2 was only

Table 4. Clinical and laboratory characteristics of ELISA1 positive and negative donors

Clinical and laboratory characteristics	ELISA1 positive N	ELISA1 positive Median (*range*) or percent	ELISA1 negative N	ELISA1 negative Median (*range*) or percent	Odds ratio[a]	95% Confidence intervals[b]	*P* value[c]
Age *years*	148	32 (10–61)	93	31 (1–65)			0.67
Gender *% male*	147	77.6%	93	60.2%	2.28	1.29–4.03	0.004
Race *% Caucasian*	147	81.6%	91	90.1%	0.49	0.22–1.09	0.08
Causes of death *% unnatural*	144	67.4%	87	59.8%	1.39	0.80–2.41	0.24
History of alcohol abuse *% yes*	63	76.2%	25	44.0%	4.07	1.53–10.85	0.004
History of drug abuse *% yes*	61	70.5%	20	20.0%	9.56	2.80–32.56	<0.001
Blood product transfusions *number*	130	2 (0–65)	84	2 (0–50)			0.92
Blood product transfusions *% yes*	130	63.9%	84	63.1%	1.03	0.58–1.83	0.91
Alanine aminotransferase levels *U/liter*	99	41 (8–476)	60	30 (3–445)			0.10
Alanine aminotransferase levels *% >100 U/liter*	99	29.3%	60	21.7%	1.50	0.71–3.18	0.29
Blood alcohol level *mg/dl*	41	100 (0–3300)	27	0 (0–404)			0.004
Blood alcohol levels *% >100 mg/dl*	41	56.1%	27	25.9%	3.65	1.27–10.53	0.01
Positive toxic screen *% positive*[d]	21	47.6%	22	0%	Infinite		<0.001
Anti-HBs *% positive*	32	31.3%	12	8.3%	5.00	0.57–44.20	0.12
Anti-HBc *% positive*	58	29.3%	81	2.5%	16.38	3.61–74.36	<0.001
Anti-CMV *% positive*	136	64.7%	79	46.8%	2.08	1.18–3.66	0.01

[a] Odds ratio of being ELISA1 positive in donors with the characteristic
[b] 95% Confidence intervals of the odds ratio
[c] Wilcoxon for continuous variables and Chi-square for discrete variables
[d] Presence of drugs with potential for abuse

Table 5. Clinical and laboratory characteristics of ELISA2 positive donors with and without HCV RNA in the serum

Clinical and laboratory characteristics	PCR positive N	PCR positive Median (*range*) *or percent*	PCR negative N	PCR negative Median (*range*) *or percent*	Odds ratio[a]	95% Confidence intervals[b]	*P* value[c]
Age *years*	60	32 (18–55)	49	30 (10–57)			0.20
Gender *% male*	60	83.3%	48	75.0%	1.67	0.65–4.28	0.29
Race *% Caucasian*	60	85.0%	49	79.6%	1.45	0.54–3.92	0.46
Causes of death *% unnatural*	59	72.9%	47	68.1%	1.26	0.54–2.92	0.59
History of alcohol abuse *% yes*	31	90.3%	13	84.6%	1.70	0.25–11.58	0.59
History of drug abuse *% yes*	28	85.7%	14	71.4%	2.40	0.50–11.54	0.27
Blood product transfusions *number*	53	2 (0–65)	43	2 (0–20)			0.86
Blood product transfusions *% yes*	53	62.3%	43	65.1%	0.88	0.38–2.04	0.77
Alanine aminotransferase levels *U/liter*	40	41 (9–327)	30	46 (8–476)			0.38
Alanine aminotransferase levels *% >100 U/liter*	40	32.5%	30	26.7%	1.32	0.47–3.77	0.60
Blood alcohol level *mg/dl*	19	258 (0–438)	12	139 (0–3300)			0.68
Blood alcohol levels *% >100 mg/dl*	19	68.4%	12	58.3%	1.55	0.35–6.94	0.57
Positive toxic screen *% positive*[d]	9	66.7%	5	60.0%	1.33	0.14–12.82	0.80
Anti-HBs *% positive*	17	35.3%	10	30.0%	1.27	0.24–6.82	0.78
Anti-HBc *% positive*	23	43.5%	16	31.3%	1.69	0.44–6.47	0.44
Anti-CMV *% positive*	55	58.2%	44	63.6%	0.80	0.35–1.80	0.58
ELISA2 optical ratio >3.0	58	98.3%	46	76.1%	17.91	2.22–144.83	<0.001
RIBA2 *% positive*	29	100%	44	93.2%	Infinite		0.15

[a] Odds ratio of being PCR positive in ELISA2 positive donors with the characteristic
[b] 95% Confidence intervals of the odds ratio
[c] Wilcoxon for continuous variables and Chi-square for discrete variables
[d] Presence of drugs with potential for abuse

55.1%. Using the ELISA2 anti-HCV test to screen organ donors would lead to wastage of organs from 44.9% of donors with a positive test. Given the prevalence of HCV infection, a moratorium on the use of non-life saving transplants from ELISA2 positive organ donors would lead to waste of organs from 1.88% of donors. One strategy to minimize wastage is to identify clinical or laboratory characteristics that differentiate anti-HCV positive donors with and without HCV RNA.

We observed differences in clinical and laboratory characteristics of anti-HCV positive cadaver donors and anti-HCV negative controls that are consistent with known epidemiologic features of populations exposed to parenterally transmitted viruses. However, these differences did not distinguish anti-HCV positive donors with and without serum HCV RNA. Some investigators have suggested that anti-HCV positive donors without history of drug abuse or homosexual lifestyle, absence of anti-HBs or anti-HBc, and normal serum alanine aminotransferase levels are at low risk of transmitting disease and could hence be used for

transplantation [9]. However, our results suggest that there are no "low-risk" anti-HCV positive cadaver organ donors.

Another strategy to minimize wastage is to develop a confirmatory test for use in donors with a positive screening test. The RIBA2 has been suggested as a confirmatory test in blood donors [20, 21, 32]. However, our results in cadaver organ donors indicate that it is not specific enough to distinguish ELISA2 positive donors with and without serum HCV RNA, and would not appreciably reduce organ wastage. Hence, newer confirmatory tests with an even greater specificity need to be developed in order to reduce organ waste.

Finally, even if a strategy is identified that would differentiate anti-HCV positive donors with and without HCV RNA, the fact remains that 2.4% of cadaver organ donors test positive for serum HCV RNA by PCR, and would not be suitable for transplantation of non-life saving organs. Recently, it has been suggested that organs from HCV RNA positive donors could be safely transplanted into HCV RNA positive recipients [33]. Long-term trials are necessary to evaluate the risk of post-transplantation liver disease in these recipients. If the safety of this practice is established, organs from donors with anti-HCV could be reserved for recipients with serum HCV RNA. This practice could potentially eliminate both the need for confirmatory testing in donors with anti-HCV and the wastage of organs from anti-HCV positive donors.

Acknowledgments

Support for this study was provided by the United Network for Organ Sharing, Richmond, VA, Dialysis Clinics Inc., Nashville, TN, American Gastroenterology Association/Smith Kline Beecham Clinical Research Award (to Dr. Pereira), American Society of Transplant Physicians/Ortho Grant-in-Aid Award in Transplantation (to Dr. Pereira), and the Nephrology Clinical Research Fellowship of New England Medical Center and St. Elizabeth's Hospital, Boston.

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Appendix

Since the ELISA1 positive donors in this study represent all the ELISA1 positive donors in the OPOs, but the ELISA1 negative controls represent only a sample of the ELISA1 negative donors, we used the following formulas to calculate the prevalence of a positive test for HCV infection (ELISA1, ELISA2, PCR) in each OPO and in the donor population:

$$\text{Test prevalence} = K/N$$

$$K = A*B + C*D$$

where "K" is the number of test positive samples, "N" is the population of the OPO, "A" is the number of ELISA1 positive samples, "B" is the ratio of the number of test positive samples to the number of ELISA1 positive samples tested, "C" is the number of ELISA1 negative samples and "D" is the ratio of the number of test positive samples to the number of ELISA1 negative samples tested.

We calculated the sensitivity, specificity, PPV and NPV of each test based on PCR in each OPO and in the donor population as follows:

$$\text{Sensitivity} = a/(a + c)$$

$$\text{Specificity} = d/(b + d)$$

$$\text{PPV} = a/(a + b)$$

$$\text{NPV} = d/(c + d)$$

where "a" is the number of test positive and PCR positive samples, "b" is the number of test positive and PCR negative samples, "c" is the number of test negative and PCR positive samples, and "d" is the number of test negative and PCR negative samples. Then "a" is calculated by multiplying the total number of test positive samples (K) by the ratio of the number of test positive and PCR positive samples to the number of test positive samples tested by PCR, and $b = K - a$. Similarly, "d" is calculated by multiplying the total number of test negative samples ($N - K$) by the ratio of the number of test negative and PCR negative samples to the number of test negative samples tested by PCR, and $c = (N - K) - d$.

We used the PPV and NPV of various strategies for screening and confirmatory testing to estimate the proportion of donors that would transmit HCV and that would be wasted. Estimates of transmission (transplantation of organs from a donor with serum HCV RNA) and waste (discard of non-life saving organs, such as kidneys and pancreas, from donors without serum HCV RNA) were calculated as follows:

$$\text{Transmission} = (1 - \text{NPV})*(1\text{-test prevalence})$$

$$\text{Waste} = (1 - \text{PPV})*(\text{test prevalence})$$

For screening strategies, test prevalence was defined as the prevalence of the test in the donor population. For confirmatory strategies, test prevalence was defined as the prevalence of the test among donors with a positive screening test.

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